Gravity sensing in oat coleoptiles: scatter in growth orientation under different *g*-conditions

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ABSTRACT

Dark-grown oat seedlings depart from the expected vertical orientation, suggesting that the coleoptile is less responsive to the lateral component of a gravitational stimulus than would be expected. This phenomenon was studied by investigating the gravitropic curvatures of oat (*Avena sativa* L. cv. Seger) coleoptiles at $1 \cdot 0g$ and over a range of longitudinally applied centripetal accelerations up to $19 \cdot 4g$. In most experiments, the plants were grown and observed at a particular *g*-level throughout the experiment. Time-lapse video recordings permitted studies of the scatter, measured as the variability of the plants' angle from the vertical (or root mean square value, RMS). The coleoptiles' heights at the end of the experiments were not significantly altered under the centrifugation. Scatter increased with plant age and decreased with increasing *g*. It decreased in an almost linear fashion as a function of the logarithm of the *g*-acceleration. In a series of experiments, the *g*-level was changed from $1 \cdot 0g$ to a higher test *g*-acceleration. The scatter was then reduced within half an hour after the *g*-transition. It is pointed out that the experiments confirm that the scatter is *g*-related but that it was not predicted quantitatively by present theories of the oat coleoptile's gravitropic response kinematics.

Key-words: gravitropism; centrifuge; scatter; reciprocity; oat; coleoptile; gravitropic response; hypergravity.

Abbreviations: scatter, variability of angular growth direction of shoots around their average direction; RMS, root mean square; *g*, standard gravitational acceleration on the surface of the earth.

INTRODUCTION

For an *orthotropic* plant organ, i.e. an organ growing in the plumb line direction, a spatial deviation from the plumb line constitutes a stimulus to its gravity sensing system. The stimulus often has been assumed to be the transverse component of the gravity vector, propor-

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tional to the sine of the angular deviation of the plant organ with respect to the plumb line (see e.g. review by Volkmann & Sievers 1979). If this component is allowed to act for a long enough time, the expected gravitropic reaction would be a bending towards the plumb line.

However, plants do not grow in perfectly vertical orientation but rather show a certain scatter in their growth direction. As an example, the scatter of *Avena* shoots of roughly 15 mm height can be about 4·5°. Deviations from the plumb line have not caused a gravitropic response although they might have been present for a very long time.

Circumnutations cannot account for the alignment 'failures' because the tilted posture is often maintained in the same direction for long periods.

The oat coleoptile's *g*-detection system is capable of responding to a test stimulation as small as $0 \cdot 001g$ (see review by Volkmann & Sievers 1979). Intuitively, it seems strange that it does not maintain the coleoptile's alignment with the plumb line better than it does. To understand this behaviour, we need to know how responsive the coleoptile is to *g*-stimulations.

In some orthotropic plants, it is sufficient to place the shoot or the main root horizontally under $1 \cdot 0g$ for less than 10–15 s to initiate a gravitropic response (Johnsson & Pickard 1979). In other cases, an effect of even shorter exposure times have been reported (Sievers 1989). It is known that the gravitropic system reacts more strongly to high *g*-stimulations than to $1 \cdot 0g$-stimulations, applied transversally for the same time (e.g. Johnsson 1965; Shen-Miller 1970). In a certain stimulus interval, the gravitropic response, the curvature, seems to obey the so-called reciprocity law (for a discussion see Eidesmo *et al.* 1991). This law states that the gravitropic response will be the same as long as the product of transverse applied acceleration and the time during which it is applied is the same.

A deviation from the plumb line of 6° corresponds to a transverse acceleration of roughly $0 \cdot 1g$. This acceleration will require a 10 times longer stimulation time to cause a bending response than the $1 \cdot 0g$ acceleration if the reciprocity law is valid. The typical stimulation time would then be of the order of some minutes (cf. numbers

given in the section above). Nevertheless, one can observe tilts of this or greater magnitude maintained for hours of coleoptile growth without the coleoptile responding by bending toward the vertical.

It was this seemingly anomalous behaviour of the oat coleoptile that we addressed experimentally. Applying a higher *longitudinal* g-acceleration to the coleoptiles could cause an increased gravitropic reaction to compensate for deviations from the plumb line. If so, the scatter of the growth direction would diminish at higher g's. As outlined above, the time for the gravitropic system to react to a change in the applied acceleration was also of interest, since it bears on the applicability of the reciprocity law in gravitropism.

MATERIAL AND METHODS

Plant material and growing medium

The plant material used was the coleoptile of oat, *Avena sativa* L., cv. Seger, purchased from Svalöf AB, Svalöf, Sweden. The soil was a planting medium (Pro-Mix A) containing 60% peat and 40% vermiculite, obtained from Premier Brands, New Rochelle, NY, USA.

Cultivation method

Seeds were grown as described by Eidesmo *et al.* (1991). Therefore, only the cultivation method and the plant growth containers (cubes) will be described below.

Seeds were surface sterilized in 2% (v/v) hypochlorite for 30 min and allowed to dry. They were then planted in the rectangular soil trays filled with planting medium. Moisture content after sterilization of the medium was 75% w/w. Each tray was covered by a plate that contained nine wells, also filled with planting medium. After the seeds were planted in the trays, one seed to a well, the tray was inserted into a cube constructed with IR-transmitting windows (filters). Seeds were oriented in the trays so that the plane of the vascular bundles was perpendicular to the plane of the recorded coleoptile movements. The cube was closed by inserting a shutter on one side making the cube light tight. The cube was placed in an incubator at $22 \cdot 5 \pm 1 \,°C$.

Mesocotyl suppression

When the plants were 59 h old they received a red light treatment to suppress mesocotyl growth (thus avoiding the possible influence of mesocotyl development on measurements of coleoptile growth). The cubes were opened, seed trays were removed, and the emerging seedlings were exposed for 10 min to red light (660 nm, of the order of $0 \cdot 025 \,W \,cm^{-2}$), after which the trays were transferred immediately to the light-tight cubes.

Application of g-accelerations

Plants were grown under different chronic g conditions on the 6 m centrifuge at the Gravitational Plant Physiology Laboratory, University City Science Center, University of Pennsylvania, Philadelphia, USA. The centrifuge has been described in earlier publications (e.g. Brown *et al.* 1976; Zachariassen *et al.* 1987) and allows the continuous recording of specimens by video cameras. Therefore, the cubes were installed in swinging centrifuge cradles, together with video recording equipment, at appropriate times and allowed to grow for a prescribed number of hours.

The desired g-acceleration (perpendicular to the centre of the cradle deck and to the long axes of the seedlings) is the resultant of the constant gravitational acceleration and of the centripetal acceleration. The latter was varied by controlling the speed of the centrifuge. The experimental design provided for g-accelerations of $1 \cdot 0$, $3 \cdot 5$, $6 \cdot 1$, $11 \cdot 0$ and $19 \cdot 4 g$.

Plants were either grown and observed at a particular g-level throughout the experiment or in some cases the g-acceleration was altered when the plants were 82 h old by a single step change. Time-lapse video recordings were made of the seedlings throughout the test.

Data acquisition

Illumination for viewing the plants was by an array of IR emitting diodes which radiate in a very narrow wavelength range centred at 890 nm; no light was emitted at any wavelength less than 800 nm. The IR light beam passed through both windows of the cube to reach the camera. In this way, coleoptiles on the video image appeared as silhouettes. Angle measurements were made from the recorded video tapes.

Video pictures were taken at time intervals which varied from experiment to experiment and depended also upon the detailed g-treatment of the plants. In most cases, pictures chosen for analyses were 20 or 30 min apart.

Data reduction and analyses

Analyses of the video pictures utilized an IBM PC, digitizing board, video recorder with freeze frame capability, digital mouse and image analysis software (JAVA Jandel) to determine the angle of the plants with respect to the vertical axis, which was defined as the local plumb line (normal to the deck of the cradle). This was accomplished by positioning a line tangent to the tip of the seedling (using the upper third of the coleoptile), calculating the angle and storing it for further processing. All tests were accomplished three times, each with eight or nine plants so that, for each measurement statistic, *n* was about 25 (except for data at $1 \cdot 0 g$ when *n* was about 100).

Data for plants in a specific run were averaged, and the mean curvatures with standard deviations and standard errors were calculated.

Scatter of angular data at a certain time was expressed with aid of the standard deviation of the angles measured at that time. The standard deviation, σ, is defined as

$$\sigma = \sqrt{\frac{\sum\limits_{i=1}^{n}(\alpha_i - \bar{\alpha})^2}{n-1}}$$

where α_i is the measured, individual angle (angular deviation from plumb line projected on the film plane), n is the number of plants and $\bar{\alpha}$ the average angle.

In the experiments to be discussed, the average angle $\bar{\alpha}$ was always close to zero and $n \geqslant 25$. Therefore, the standard deviation, σ, can be approximated by the so-called RMS (root mean square) value:

$$\alpha_{RMS} = \sqrt{\frac{\sum\limits_{i=1}^{n}\alpha_i^2}{n}}$$

The deviation of angles around its average value, close to zero as mentioned above, can conveniently be called 'scatter' and expressed by either σ or α_{RMS}. The results given in the present paper are given as RMS-values in most cases.

RESULTS

Movements of individual plants

The framed inserts of Fig. 1 show drawings from two video picturs of plants in the IR-transmitting cubes from two different runs. The plants in Fig. 1a have experienced 1·0*g* throughout the growth, those in Fig. 1b have grown under 19·4*g*. Visual inspection reveals that higher *g*-levels produce a straighter growth than 1·0*g*.

The curves provide examples of the type of data which was obtained from individual plants as shown in the inserts. The curve in Fig. 1a shows the deviation of one control plant grown continuously at 1·0*g*. Note that the deviations become larger as the plant ages.

The curve in Fig. 1b provides a corresponding example of a plant grown continuously under 19·4*g*. The angle of the plant varied about 2–3° (2·35 ± 1·6) and the deviation did not seem to be affected by age in this case.

It cannot be excluded that changes in plant behaviour over time could be due to 'acclimatization'; coleoptile responsiveness might be diminished by habituation so that older plants would be less responsive to changes of the *g*-level. Figure 1c shows the effect of a momentary increase of the *g*-level on a plant at 82h. Soon after the *g*-acceleration was increased from 1·0 to 19·4*g*, a notable change in the angle of the plant toward the
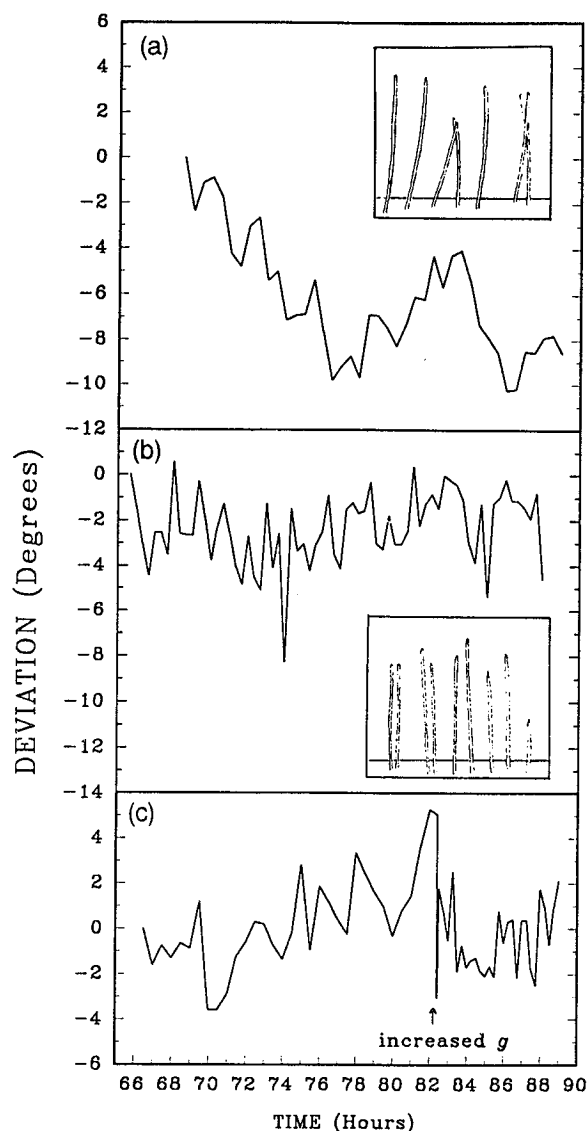

Figure 1. Angular deviation from plumb line (α) for individual plants as measured directly from video pictures and plotted as functions of time: (a) deviation of a control plant grown under 1·0*g*; (b) same as (a), but for a plant grown under 19·4*g* (inserts show plant silhouettes from cubes at the respective *g*-acceleration), (c) deviation as a function of time for a plant grown under 1·0*g* up to an age of 82h and then transferred to 19·4*g*.

plumb line was evident. There was no evidence of an effect of habituation.

Scatter as a function of time

Scatter increased with plant age, as demonstrated in Fig. 2a for 1·0, 3·5 and 19·4*g*. Scatter measured at the start of the experiment was high, perhaps because curvatures were more difficult to measure on very small plants (of the order of 1mm). For 1·0*g* plants (controls) between 68–78h, the scatter was about 4·5° (4·38 ± 0·6).
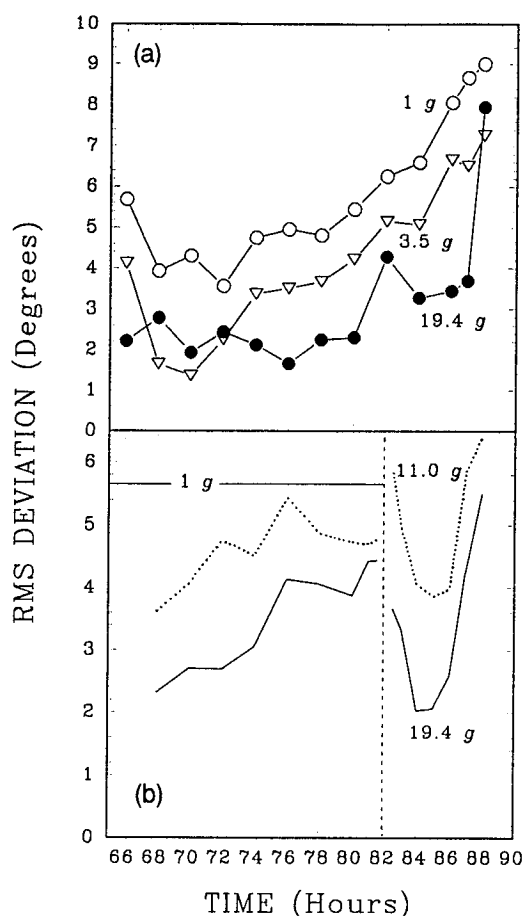
Figure 2. (a) Scatter as a function of time for plants grown at
1·0g (○), 3·5g (▽) and 19·4g (●). The scatter was measured as
the root mean square deviation from the plumb line. Lengths of
coleoptiles at the end of the experiment were: 20·8 ± 1·5 mm
(1g), 20·7 ± 2·8 mm (3·5g), 20·0 ± 2·7 mm (6·1g), 21·7 ± 0·4 mm
(11·0g) and 18·7 ± 2·1 mm (19·4g). (b) Scatter as a function of
time for plants grown under 1·0g (up to 82 h) and then
transferred to high g conditions: (—) 19·4g; (····) 11·0g.
(Average of three different runs at each g-level.)

Scatter increased with plant age. At around 86 h, a
rapid increase in the scatter is evident (see also
'Discussion').

Plants grown at 19·4g were straighter and less variable
than the 1·0g controls. The data in Fig. 2a show that the
scatter was about 2·2° for plants 68–80 h old. The scatter
increased slowly until the plants reached 87 h at which
time it increased abruptly. At the higher g-level, the
older seedlings tended to buckle. This was very likely
due to the mechanical effects of the g-acceleration, and
therefore, related to the greater height (and mass) of the
plants.

For g-values in between these two extremes, some
observations are relevant.

The 3·5g curve is situated in between the 1·0 and
19·4g curves for most of the experimental period. For

young coleoptiles (less than 72 h), the scatter was
roughly the same as for 19·4g plants, i.e. lower than for
1·0g plants. Plants grown at 6·1g showed a slight
increase in scatter throughout the experimental period
(data not shown). It was about 2·5° at 68–80 h. The data
from the 11·0g plants showed a scatter very similar to
that of the 19·4g plants.

Lengths of coleoptiles and mesocotyls were evaluated
at the end of the experimental period (hour 89–90) and
compared for different gs and treatments. There were
no significant differences at the 1% level between the
heights of the 1·0g plants and the others.

Scatter of plants subjected to step changes in the applied g-acceleration

In a series of experiments, plants were transferred at 82 h
of age from 1·0g condition to higher g-values. Figure 2b
shows results from such experiments. The scatter before
the change in g increased gradually with time (as for all
control plants, cf. Fig. 2a). The response to the change
was a rapid reduction in scatter (after a transient
period), as exemplified for 19·4 and 11·0g conditions in
Fig. 2b. The rapid reactions were seen also in individual
plants. An example is shown in Fig. 1c: they occurred
within a 30-min period.

Towards the end of the experiment, the scatter again
increased rapidly, as always for high g-values (cf. 19·4g
results in Fig. 2a).

Scatter as a function of g-value

Figure 3 demonstrates the relationship between scatter
and the chronically applied acceleration. Data for plants
79–80 h old are compared with data for plants 84–85 h
old. The plots show that the consistent displacement of
the older plants was greater than that of younger plants.
The effect is more prominent at the lower g-level. It is
evident that the scatter decreased when acceleration
increased. Scatter was almost linearly related to the
logarithm of the g-level for plants of a given age.

For young plants (up to about 70 h), the scatter was
almost independent of the g-level (cf. Fig. 2a).
However, 1·0g plants tended to exhibit higher scatter
than plants which experienced other g-levels. The small
g-dependence for young plants could be accounted for if
very young (small) plants were less responsive to
tropistic stimulations, but it should also be stressed that
the curvatures were difficult to measure at this stage as
mentioned above.

DISCUSSION

Alerted by mostly qualitative observations indicating
prevalent failure of dark grown oat seedling shoots to
consistently maintain a vertical position, we have exam-
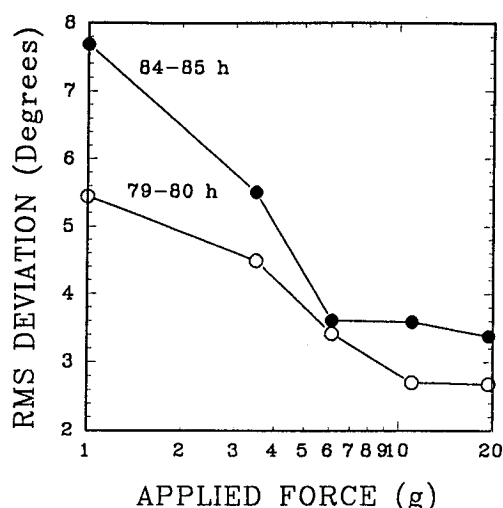ined the gravitropic scatter around the vertical shown by

Figure 3. Scatter as a function of the applied acceleration. Scatter was expressed as RMS-deviations and the accelerations under which the plants were growing is plotted on the logarithmic x-axis: (○) data for plants of age 79–80 h; and (●) data for plants of age 84–85 h.

seedlings grown at different g-levels. It was found that an increase in the g-value decreased the scatter in a systematic way: the relationship was non-linear. A plot of scatter against the log of the g-level was nearly linear. That is a functional dependence found in several physiological sensory systems (Weber-Fechner's law; see e.g. Rawitscher 1932; Bentrup 1979). Accordingly plants grown at the higher g-levels grew straighter than those at $1\cdot0g$ (Figs 2a & 3).

Extrapolating the data of Fig. 3 to g-values lower than unity (notoriously dangerous, but irresistible) certainly suggest that plants would increase their scatter under such circumstances. From Fig. 3, an extrapolated value of the order of 10° is obtained for $10^{-3}g$, a g-value which is of interest in life science space research. The extrapolation is based on the 79–80 h data from Fig. 3 (the 84–85 h data would give a slightly higher extrapolated value). If we had chosen linear scales (instead of the semi-logarithmic diagram in Fig. 3), the extrapolation procedure would indicate a zero-g scatter of 6 and 9° for young and old plants, respectively. Therefore, both extrapolation procedures predict that experiments in weightlessness will give rise to higher scatter in the plant material.

The gravitropic response system of the oat seedlings has been studied extensively and it has been well established that the coleoptile can detect and respond to a unit g lateral stimulus applied for as little as 10–15 s (Johnsson & Pickard 1979). Nevertheless, our results show that scatter was often much greater than would be predicted by the observed departure from the plumb line. Two possible causes for scatter are circumnutations and so-called spontaneous curvatures.

Nutational movements are ubiquitous and studies of the growth directions of orthotropic plants must also take them into consideration; nutations interact with gravitropic movements (Britz & Galston 1982; see also Johnsson 1979; Brown 1991). It might be that tendencies for circumnutations can be present in some of our data. However, regular circumnutations with clear-cut period and constant amplitude do not seem to be present: irregular, spontaneous movements dominate the picture and will contribute to the scatter. Further analysis of details of the spontaneous growth movements can be worthwhile to find hidden, small amplitude periodicities.

Spontaneous deviations also occur during growth (Larsen 1957; Johnsson 1966), causing a scatter in the average vertical direction. If such spontaneous, random curvatures were allowed to develop without any correction (as in weightlessness), one could expect the overall scatter to increase with time in a predictable manner. It has been argued (Johnsson 1966) that the mean squared value of the individual angles should increase linearly with time under such circumstances. For the time being, we cannot rule out the possibility that the increased scatter in the present experiments is due to spontaneous growth processes.

In the present study, it was also demonstrated that a plant which at $1\cdot0g$ shows a deviation from the vertical will start compensating for this deviation very soon after it has been transferred to a higher g. The response raises an important question: Why is scattering diminished so rapidly (and at all) after a step transfer to high gs?

The gravitropic stimulus is normally considered to be a function of the product of the stimulating g-acceleration and stimulation time. Since the plants react to the deviation from the plumb line within about 30 min at $11\cdot0g$ (see Fig. 2b), they should, if the product of acceleration and time constitutes the relevant stimulus, have shown a response within 11×30 min at $1\cdot0g$. This product corresponds to roughly $5g \times$ hours, which the plants in $1\cdot0g$ have exceeded after 5 h, evidently without having compensated as effectively as under $11\cdot0g$ conditions.

The simple dose concept, based on the product of time and acceleration cannot explain these facets of the gravitropic system. The transfer to high g conditions produced a reaction (Fig. 2b), while the long time at $1\cdot0g$ did not. Therefore, the response is of a complicated, non-linear type, and the simple concept of reciprocity is violated in these gravitropic experiments on the oat coleoptile.

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